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ORIGINAL RESEARCH

Mechanism of microRNA-152-3p-Mediated Regulation of Autophagy and Sensitivity in Paclitaxel-Resistant Ovarian Cancer Cells

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Objective: The study investigated microRNA-152-3p-mediated autophagy and sensitivity of paclitaxel-resistant ovarian cancer cells.

Methods: The miR-152-3p mimics and miR-152-3p inhibitor were transfected in A2780 cells and A2780T cells, and the scrambled sequences were transfected as a negative control group, the transfection efficiency was detected by qPCR technology. MTT was used to detect the proliferation and IC50 value of the cells after transfection. The expression of target proteins in A2780 cells and A2780T cells were detected by qPCR; The expression of phosphatase and tensin homolog (PTEN) and ATG4D after transfection were analyzed by Western blot. The knockdown efficiency of PTEN was detected by reverse qRT-PCR, MTT and Western blot.

Results: The expression level of miR-152-3p in A2780T cells was 52-fold higher than that in A2780 cells according to the results of qPCR. Downregulation of miR-152-3p reversed PTX-induced autophagy, inhibited cell proliferation and apoptosis, and reduced drug resistance in A2780T cells. Moreover, PTEN appeared to be a potential target of miR-152-3p, and low expression levels of miR-152-3p increased PTX sensitivity by downregulating PTEN in vitro.

Conclusion: PTEN may be a novel therapeutic target gene for patients with PTX-resistant ovarian cancer. These findings provide a potential translational framework for developing novel therapeutic strategies to overcome paclitaxel resistance in ovarian cancer.

Keywords: microRNA-152-3p, ovarian cancer, paclitaxel resistance, autophagy, PTEN

Introduction

Ovarian cancer ranks as one of the most fatal malignancies affecting women worldwide,[1] with chemotherapy being the cornerstone of treatment. Despite paclitaxel's status as a first-line treatment, resistance is common,[2] often leading to relapse and increased mortality.[3] This highlights the urgent need to elucidate the mechanisms underlying paclitaxel resistance in ovarian cancer.

Autophagy, a process central to cellular homeostasis,[4–6] plays contrasting roles in cancer.[7] Initially, it serves as a tumor suppressor, preventing oncogenic transformation and shielding healthy cells from malignant progression.[8] Yet, as cancer advances, autophagy can support tumor growth and survival, contributing to therapeutic resistance and tumor resilience. This dualistic nature makes autophagy a compelling target for cancer therapy, particularly in overcoming drug resistance.[9]

Recent studies underscore the importance of autophagy in the context of chemotherapy resistance. For instance, Zhang et al reported that TXNDC17-mediated regulation of BECN1 induces autophagy, conferring paclitaxel resistance in ovarian cancer.[10] Furthermore, interactions between PTEN and autophagy-related proteins like BECN1 have been

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implicated in the resistance mechanisms of epithelial ovarian cancer.[11–13] Moreover, recent research has demonstrated that PTEN interacts with autophagy-related pathways, which may contribute to cancer cell survival and chemoresistance.[14] The modulation of autophagy by miRNAs, including their influence on drug resistance and cancer progression, is increasingly recognized. For example, Xu et al demonstrated that miR-541 sensitizes hepatocellular carcinoma to sorafenib through the ATG2/RAB1B axis.[15] Similarly, Huang et al found that *miR-93* regulates multiple autophagy regulators, affecting glioma resistance to temozolomide.[16]

Notably, miR-152-3p has been implicated in the proliferation and metastasis of thyroid cancer cells, including the modulation of ERBB3 expression.[17] However, the precise mechanisms by which miR-152-3p modulates autophagy-related resistance to paclitaxel in ovarian cancer remain uncharted. Our study aims to fill this gap by exploring the role of miR-152-3p in paclitaxel resistance, particularly its regulatory effects on PTEN and ATG4D, which may be pivotal in autophagy-mediated drug resistance pathways in ovarian cancer cells.

Materials and Methods

Bioinformatic Analysis

Expression data for miR-152-3p was retrieved from GEO datasets (GSE53829 and GSE190245) and TCGA ovarian cancer cohort. Putative miR-152-3p targets were identified through an integrated analysis of multiple prediction algorithms: miRDB (v6.0), TargetScan (v7.2), and miRWalk (v3.0). These predictions were cross-referenced with autophagy-related genes from the Human Autophagy Database (HADb). Based on experimental validation of elevated miR-152-3p expression in paclitaxel-resistant cells, we prioritized ATG4D and PTEN for functional studies after confirming their significant correlation ($|r| > 0.1$, StarBase v3.0) with miR-152-3p expression.

Cell Culture

The human ovarian cancer cell line A2780 and its paclitaxel-resistant variant A2780T were obtained from Shanghai Mei Xuan Biotechnology Co., Ltd. (Shanghai, China). The parental A2780 cells were maintained in RPMI-1640 medium (Invitrogen; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc). To maintain the drug-resistant phenotype, A2780T cells were cultured in the same medium with the addition of paclitaxel (PTX; Sigma-Aldrich). The PTX concentration was gradually increased from 100 ng/mL to 400 ng/mL to achieve approximately 20-fold resistance compared to the parental cells. All cells were maintained at 37°C in a humidified atmosphere containing 5% CO2.

MTT Assay

Cell viability was determined by MTT assay (Sigma-Aldrich; Merck KGaA). The cells were seeded into 96-well plates (1x104 cells/mL) for 24 h. Based on both pre-experimental results and prior research findings,[18,19] A2780/A2780T cells were treated with paclitaxel at a concentration gradient of 20, 15, 10, 5, 2.5 μg/mL. A2780/A2780T cells were treated with paclitaxel at a concentration gradient of 20,15,10, 5, 2.5 μg/mL. After 48 h, 50 μL MTT (Sigma-Aldrich; Merck KGaA) was added to each well and incubated for additional 4 h. Finally, dimethyl- sulfoxide (150 μL) was added to each well, and the absorbance of the cells at 570 nm was measured using a microplate reader (SpectraMax, Molecular Devices). Cell viability was calculated as the absorbance of treated cells relative to that of untreated cells.

Reverse Transcription-Quantitative PCR (RT-qPCR)

Total RNA was extracted from A2780T and A2780 cells using Trizol (Shanghai GenePharma Co., Ltd.) according to the the manufacturer's instructions. Reverse transcription of RNA to cDNA was performed with EasyScript One-Step Removal and cDNA Synthesis SuperMix (TransGen Biotech, China). The expression levels of mature miR-152-3p and the mRNA levels of PTEN and ATG4D were quantified by RT-qPCR employing SYBR Green Master Mix (TransGen Biotech, China). The thermocycling conditions were as follows: Initial denaturation at 94°C for 30s, followed by 45 cycles of 94°C for 5 s and 60°C for 30s. U6 was used as the internal control for normalization of miR-152-3p,

Table 1 The Primers Used in qRT-PCR

Gene name	Primer Sequences
miR-152-3p	Forward: 5′-TCCGCTCAGTGCATGACAG-3′
	Reverse: 5′-TATGGTTGTTCACGACTACTTCAC-3′
PTEN	Forward: 5′-TGGATTCGACTTAGACTTGACCT-3′
	Reverse: 5′-GCGGTGTCATAATGTCTCTCAG-3′
ATG4D	Forward: 5′-CGCTAGTGGCACACATCCTC-3′
	Reverse: 5′-TCACGCAGGGCACATACAC-3′
GAPDH	Forward: 5′-CGACAGTCAGCCGCATCTT-3′
	Reverse: 5′-CCAATACGACCAAATCCGTTG-3′
U6	Forward: 5′-CGCTTCGGCAGCACATATAC-3′
	Reverse: 5′-TTCACGAATTTGCGTGTCATC-3′

while GAPDH served as the internal control for normalization of mRNAs expression. The $2^{-\Delta\Delta Ct}$ method was used to quantify the mRNA level. The primers used in RT-qPCR are shown in [Table 1].

Cell Transfection

A2780 and A2780T cells were transfected using Lipofectamine 2000 (Invitrogen, USA) according to manufacturer's protocol. miR-152-3p mimics, inhibitors, and their respective controls (double-stranded NC mimic and single-stranded NC inhibitor) were obtained from GenePharma. Cells were seeded at 3.0×105 cells/mL in six-well plates and transfected at 60–70% confluency with 100 nM of the oligonucleotides. Briefly, transfection complexes were prepared by combining oligonucleotides with Lipofectamine 2000 in serum-free medium and incubated for 20 min at room temperature. Following 6 h of transfection, the medium was replaced with complete growth medium. Cells were cultured for an additional 24 h before RNA or protein extraction.[20] Transfection efficiency was validated by qRT-PCR. Oligonucleotide sequences are listed in [Table 2].

Transmission Electron Microscopy

Cells (A2780 and A2780T) were treated with paclitaxel (400 ng/mL) for 24 hours, reaching a density of 80–90%. The treated cells were fixed in 2.5% glutaraldehyde at 4°C for 24 hours, followed by three washes with phosphate-buffered saline (PBS). Next, the samples were fixed in 1% osmium tetroxide for 2 hours, dehydrated through a graded ethanol series, and embedded in epoxy resin 618. Ultrathin sections (~50 nm) were obtained using an ultramicrotome and stained with 3% uranyl acetate and lead citrate which are widely used contrast agents compatible with epoxy resin-embedded samples. The samples were examined under a transmission electron microscope (JEM-1200EX; JEOL, Ltd.) at varying magnifications (×10,000, ×25,000, ×200,000). Autophagosomes were identified as double-membrane structures containing engulfed cellular materials such as mitochondria or endoplasmic reticulum fragments.[21]

Table 2 RNA Oligo Synthesis

Gene name	Primer Sequences
miR-152-3p mimic	Sense: 5′-UCAGUGCAUGACAGAACUUGG-3′
	Antisense: 5′-AAGUUCUGUCAUGCACUGAUU-3′
NC mimic	Sense: 5′-UUCUCCGAACGUGUCACGUTT-3′
	Antisense: 5′-ACGUGACACGUUCGGAGAATT-3′
si PTEN	Sense: 5′-CGGGAAGACAAGUUCAUGUTT-3′
	Antisense: 5′-ACAUGAACUUGUCUUCCCGTT-3′
NC	Sense: 5′-UUCUCCGAACGUGUCACGUTT-3′
	Antisense: 5′-ACGUGACACGUUCGGAGAATT-3′
miR-152-3p inhibitor	Sense: 5′-CCAAGUUCUGUCAUGCACUGA-3′
NC inhibitor	Sense: 5′-CCAAGUUCUGUCAUGCACUGA-3′

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Flow Cytometry

Cell apoptosis was examined by flow cytometry using annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) staining according to the manufacturer's instructions (KeyGEN BioTECH, KGA108). Briefly, cells were collected at the prespecified time points, washed twice with cold PBS and centrifuged at 4°C and 1,000 x g for 5 min. Next, cells were resuspended in 1X Annexin V Binding Buffer for 15 min. Subsequently, 5 μL Annexin V-FITC was added to 100 μL cell suspension, and 15 min later, 5 μL PI was added for fluorescence-activated cell sorting (FACS) analysis in a FACS Calibur flow cytometer (BD Biosciences).

Western Blot

Western blot analysis was performed as described previously.[22] In brief, cells were rapidly harvested using a cell scraper, followed by centrifugation in a high-speed refrigerated centrifuge at 4°C and 12,000 x g for 15 min. The supernatant was removed, and the total protein concentration was determined using the BCA protein assay kit. Proteins (20 μg per lane) were then loaded into each lane and separated using 10% SDS-PAGE gels. After separation, proteins were transferred to 0.45 μm PVDF membranes and blocked with 5% non-fat milk for one hour at room temperature. The membranes were subsequently incubated with primary antibodies overnight at 4°C. The primary antibodies used in this study are as follows: P Glycoprotein / ABCB1 antibody (WL02395, Wanleibio Co., Ltd)., GSTP1 antibody (66715-1-Ig, Proteintech), Bax antibody (#2772, Cell Signaling Technology), Bcl-2 antibody(#3498, Cell Signaling Technology), LC3 α/β antibody (WL01506, Wanleibio Co., Ltd)., p62 antibody (AF5384, Affinity), PTEN antibody (WL01901, Wanleibio Co., Ltd)., ATG4D Polyclonal Antibody (ABP56793, Abbkine), MRP1 antibody (#72202, Cell Signaling Technology), ABCG2 antibody (#42078, Cell Signaling Technology), anti-GAPDH antibody (ab181602, Abcam). The next day, the membranes were incubated with appropriate secondary antibodies at room temperature for 1 h and the signals were imaged by ChemiDoc XRS+ system (Bio-Rad, USA). Grey scale values of each blot were measured by ImageJ software, and the intensity of each band was normalized to the loading control GAPDH.

Statistical Analysis

Statistical analyses were conducted using GraphPad Prism software (version 8.2.1.1; GraphPad; Dotmatics). Data normality was evaluated using the Shapiro–Wilk test. For comparisons between two groups, unpaired Student's *t*-test was used for normally distributed data. For multiple group comparisons, one-way ANOVA was performed, followed by Dunnett's *t*-test for comparisons against a single control group or Fisher's protected least significant difference (PLSD) test for pairwise comparisons among all groups. The threshold for statistical significance was set at $p < 0.05$, reflecting a widely accepted standard for balancing the risks of Type I and Type II errors. Adjustments for multiple comparisons were applied where necessary to minimize false-positive results.

Sample sizes were calculated based on preliminary experiments to achieve 80% statistical power at $\alpha = 0.05$, considering expected effect sizes and variability. Data are presented as mean ± standard deviation (SD) from at least three independent experiments unless otherwise stated. For correlation analyses, Pearson's correlation coefficient was calculated, with significance set at $p < 0.05$. Spearman's rank correlation was applied for non-normally distributed data, where applicable.

Ethics Statement

This study was exempt from ethical review according to Article 32 (items 1 and 2) of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects (National Health Commission of China, February 18, 2023), as it utilized existing publicly available data that was previously collected with appropriate ethical approvals and patient consent.

Results

miR-152-3p Is Upregulated in Ovarian Cancer Tissues and Is Associated With Ovarian Cancer Resistance to PTX

It has been suggested that miRNAs may regulate autophagic activity by directly targeting autophagy-related proteins or pathways.[23] Based on literature reviews and databases, the present study screened various miRNAs that were reported to

be differentially expressed in ovarian cancer tissues compared to normal tissues. To investigate miRNAs involved in ovarian cancer autophagy regulation, we performed differential expression analysis using the GSE53829 dataset (14 normal ovarian and 27 cancer tissue samples). Using selection criteria of $P < 0.05$ and $|\log2FC| > 0.5$, we identified 639 differentially expressed miRNAs, comprising 248 upregulated and 381 downregulated miRNAs [Figure 1A]. Notably, miR-152-3p showed significant upregulation in ovarian cancer tissues compared to normal controls [Figure 1B]. To explore the relationship between miR-152-3p and paclitaxel resistance, we analyzed the GSE190245 dataset, which includes expression profiles of parental A2780 cells and their paclitaxel-resistant derivatives maintained at 300 or 1,100 ng/mL PTX. Using stringent criteria ($P < 0.05$, $|\log2FC| > 1$), we identified consistently differentially expressed miRNAs across both PTX concentrations. miR-152-3p emerged as significantly upregulated in PTX-resistant cells compared to parental cells [Figure 1C and D]. These findings demonstrate miR-152-3p upregulation in both ovarian cancer tissues and PTX-resistant cells, suggesting its potential role in paclitaxel resistance.

miR-152-3p Expression Correlates With Tumor Size in Ovarian Cancer

To evaluate the clinical significance of miR-152-3p in ovarian cancer, we analyzed miRNA expression profiles and clinical data from 424 ovarian cancer patients in the TCGA database. Patients were stratified into high and low expression groups based on median miR-152-3p levels. Statistical analysis revealed a significant correlation between miR-152-3p expression and tumor volume [Table 3].

A2780T Is a PTX-Resistant Ovarian Cancer Cell Line

PTX-resistant ovarian cancer cells were purchased directly from Shanghai Meixuan Biotechnology Co., Ltd. First, any morphological differences between A2780T and the parental cell line A2780 were assessed, and no significant difference between the two cell lines was found [Figure 2A]. Next, the half-maximal inhibitory concentration (IC_{50}) of A2780T and A2780 cells was determined via MTT assay, and the IC_{50} value of A2780T cells was significantly higher than that of A2780 cells [Figure 2B and C]. P-glycoprotein (P-gp), encoded by the ABCB1 gene, is key in cancer drug resistance, expelling chemotherapeutic drugs from cells and lowering their effectiveness.[24] It binds drugs and ATP to promote drug efflux, leading to reduced drug levels within cells and resistance. P-gp is prevalent in drug-resistant cancer cells.[25,26] Thus, the expression of *P-gp* in A2780T and A2780 cells was determined. Compared with A2780 cells, P-gp was highly expressed in A2780T cells [Figure 2D and E]. The results demonstrated that A2780T were PTX-resistant ovarian cancer cells.

miR-152-3p enhances proliferation and PTX resistance while suppressing apoptosis in To validate our bioinformatics findings, we first compared miR-152-3p expression between A2780 and A2780T cells. qRT-PCR analysis revealed a 52-fold higher expression in A2780T cells compared to A2780 cells [Figure 3A]. To investigate whether miR-152-3p modulation affects PTX sensitivity in A2780T cells, cells were transfected with miR-152-3p mimic, miR-152-3p inhibitor or miR-NC, followed by PTX treatment. RT-qPCR was used to verify the transient transfection efficiency of miR-152-3p [Figure 3B and C]. To explore the functional role of miR-152-3p in ovarian cancer, MTT assay was employed to assess the proliferation of A2780T and A2780 cells after transfection. As expected, low expression of miR-152-3p inhibited cell proliferation [Figure 3D and E]. In drug sensitivity assays, miR-152-3p inhibition reduced the IC50 value of A2780T cells by approximately 50%, while its overexpression increased the IC50 value of A2780 cells by about 60% [Figure 3F–I]. Western blot analysis showed that miR-152-3p inhibition decreased expression of drug-resistance markers, with GSTP1 and P-gp levels reduced by 28% and 61%, respectively. [Figure 3J–L]. Conversely, miR-152-3p overexpression elevated these markers in A2780 cells, although notably, P-gp was undetectable in parental A2780 cells [Figure 3M and N].

Flow cytometry analysis of cell death revealed that miR-152-3p inhibition enhanced PTX-induced apoptosis and secondary necrosis in A2780T cells, as indicated by increased Annexin V-positive/PI-negative and Annexin V/PI double-positive populations, respectively [Figure 4A and B]. In A2780 cells, overexpression of miR-152-3p by transfection with miR-152-3p mimics decreased PTX-induced apoptosis. [Figure 4C and D].

Furthermore, the expression of Bax, Bcl-2 were detected by Western blot. The results showed that, when miR-152-3p was suppressed, the expression of Bax increased and was accompanied by a decrease in Bcl-2 expression. The survival advantage of

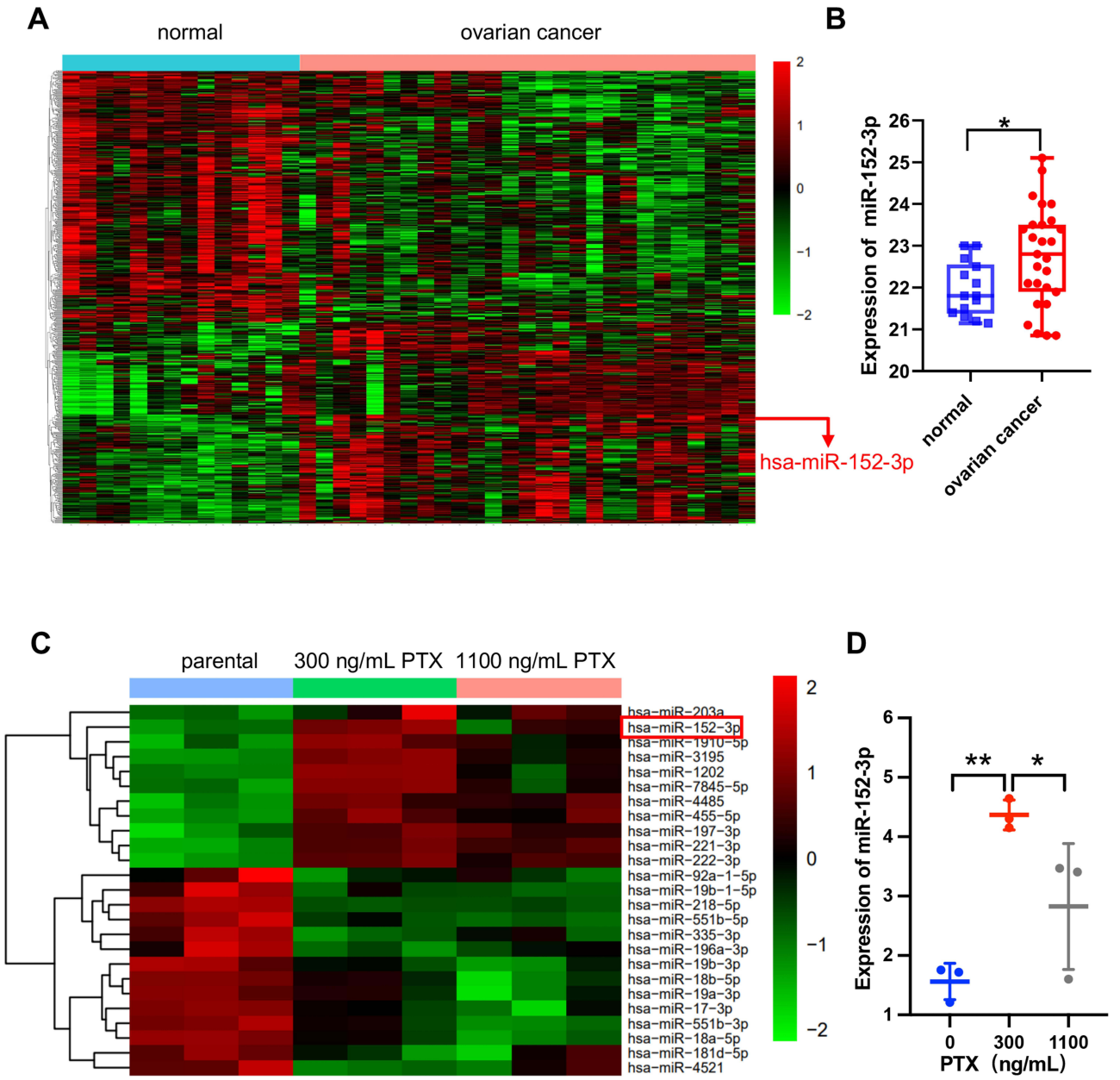

Figure 1 miR-152-3p is upregulated in ovarian cancer tissues and PTX-resistant cells (**A**) Heatmap showing differential miRNA expression between normal ovarian tissues (n = 14) and ovarian cancer tissues (n = 27) from GSE53829 dataset. A total of 639 differentially expressed miRNAs were identified ($P < 0.05$, |log2FC| > 0.5). (**B**) Box plot showing the significant upregulation of miR-152-3p in ovarian cancer tissues compared to normal tissues (*$P < 0.05$). (**C**) Heatmap depicting 25 differentially expressed miRNAs (11 upregulated and 14 downregulated) between parental A2780 cells and PTX-resistant cells maintained at 300 ng/mL or 1,100 ng/mL PTX from GSE190245 dataset ($P < 0.05$, |log2FC| > 1). (**D**) Box plot showing the progressive upregulation of miR-152-3p in PTX-resistant cells compared to parental cells (**$P < 0.01$ vs 0 ng/mL; *$P < 0.05$ vs 300 ng/mL). Data are presented as mean ± SD.
Abbreviations: miR, microRNA; PTX, paclitaxel.

resistant cells under paclitaxel treatment is reinforced by these protein expression changes, which suggest a shift toward anti-apoptotic mechanisms.[27] [Figure 4E–G]. However, after overexpression of miR-152-3p in A2780, the opposite trend was shown [Figure 4H–J]. These results indicated that miR-152-3p was capable of sensitizing ovarian cancer cells to paclitaxel treatment. In addition, the knockdown of miR-152-3p inhibited proliferation and promoted apoptosis of paclitaxel-resistant ovarian cancer cells.

Table 3 Relationship Between miR-152-3p Expression and Clinicopathological Characteristics of Ovarian Cancer Patients

Characteristics	miR-152-3p Expression		Total (N=424)	a P value	FDR
	High (N=212)	Low (N=212)			
Clinical Stage				0.5	0.96
Stage IIA	2 (0.47%)	1 (0.24%)	3 (0.71%)		
Stage IIB	3 (0.71%)	1 (0.24%)	4 (0.94%)		
Stage IIC	6 (1.42%)	9 (2.12%)	15 (3.54%)		
Stage IIIA	2 (0.47%)	4 (0.94%)	6 (1.42%)		
Stage IIIB	5 (1.18%)	11 (2.59%)	16 (3.77%)		
Stage IIIC	156 (36.79%)	154 (36.32%)	310 (73.11%)		
Stage IV	38 (8.96%)	32 (7.55%)	70 (16.51%)		
Sample type				0.28	0.85
Primary Tumor	206 (48.58%)	210 (49.53%)	416 (98.11%)		
Recurrent Tumor	6 (1.42%)	2 (0.47%)	8 (1.89%)		
Tumor size				0.0059**	0.02*
No macroscopic disease	39 (9.20%)	56 (13.21%)	95 (22.41%)		
1–10 mm	102 (24.06%)	113 (26.65%)	215 (50.71%)		
11–20 mm	22 (5.19%)	8 (1.89%)	30 (7.08%)		
>20 mm	49 (11.56%)	35 (8.25%)	84 (19.81%)		
Vital status				0.48	0.96
DECEASED	139 (32.78%)	131 (30.90%)	270 (63.68%)		
LIVING	73 (17.22%)	81 (19.10%)	154 (36.32%)		

Notes: $^{**}P < 0.01$, $^{*}P < 0.05$, a Chi-square test.

PTX Induces Autophagy in PTX-Resistant Ovarian Cancer Cells

The ability of PTX to induce autophagy has been reported in the literature.[28] The present study further investigated whether PTX induces autophagy in A2780T cells. In the process of autophagy induction, the non-lipid form of LC3 (LC3-I) is combined with phosphatidylethanolamine and then converted into the lipid form of LC3 (LC3II), which is associated with the biogenesis of autophagosomes.[29] In addition to LC3II, the receptor SQSTM1 (p62) is also used as a marker for autophagy induction, since p62 protein binds to LC3 and the ubiquitinated substrate is degraded during autophagy.[30] As LC3II is easily degraded on the lysosome, the expression of LC3II and p62 in the presence or absence of the lysosomal inhibitor chloroquine (CQ) was detected. It was found that, when the cells were treated with different concentrations of PTX, the expression of LC3II in A2780T cells increased significantly, while the expression of p62 decreased, and in the presence of CQ, the expression of LC3II and p62 both increased, suggesting autophagy induction occurs [Figure 5A–C, G, I and J]. There were no obvious changes in A2780 cells. [Figure 5D–F, H, K and L]. Transmission electron microscopy (TEM) images revealed the presence of autophagosomes in both A2780 and A2780T cells, with distinct differences in their abundance and morphology upon treatment with paclitaxel (PTX, 400 ng/mL for 24 hours). Autophagosomes were identified by their characteristic double-membrane structures and enclosed contents, such as fragmented mitochondria and endoplasmic reticulum. In A2780T cells, TEM analysis suggested an apparent increase in the number of autophagosomes in A2780T cells compared to A2780 cells [Figure 6].These data suggested that upon PTX induced autophagy in upon PTX -resistant ovarian cancer.

Knockdown of miR-152-3p Inhibits PTX-Induced Autophagy in A2780T Cells, and PTEN, ATG4D are Target Genes of miR-152-3p

The present study further validated the effects of miR-152-3p on autophagy in A2780T cells. The expression of miR-152-3p in the A2780T cells was knocked down by transfection, and the expression of LC3II and p62 was detected by Western blot. Compared with the NC group, the LC3II expression decreased, while the expression of p62 increased after

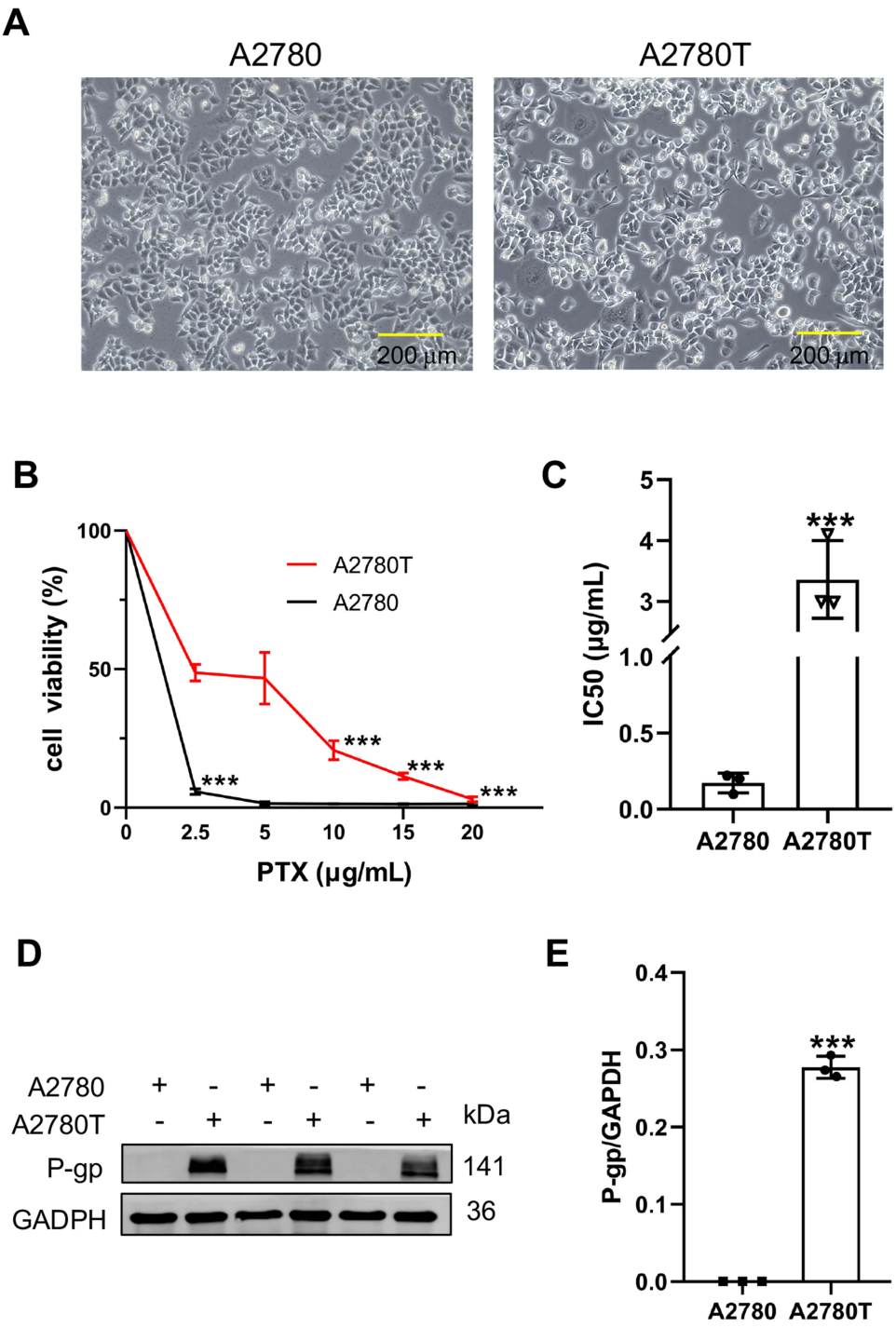

Figure 2 Characterization of paclitaxel-resistant ovarian cancer cells (A2780T). (**A**) Representative phase-contrast microscopy images showing morphology of A2780 and A2780T cells. (**B** and **C**) Cell viability and IC50 values of A2780 and A2780T cells determined by MTT assay. (**D** and **E**) P-glycoprotein expression levels analyzed by Western blot, normalized to GAPDH. Data are presented as mean ± SD. ***$P < 0.001$ vs A2780 cells.

transfection of miR-152-3p inhibitor [Figure 7A–C]. It is worth noting that the expression levels of LC3II and p62 were reduced by treatment with 400 ng/mL PTX, indicating decreased autophagic activity. The above experiments showed that inhibition of miR-152-3p suppressed the autophagy of A2780T cells, suggesting that miR-152-3p functions as a positive regulator of autophagy in A2780T cells.

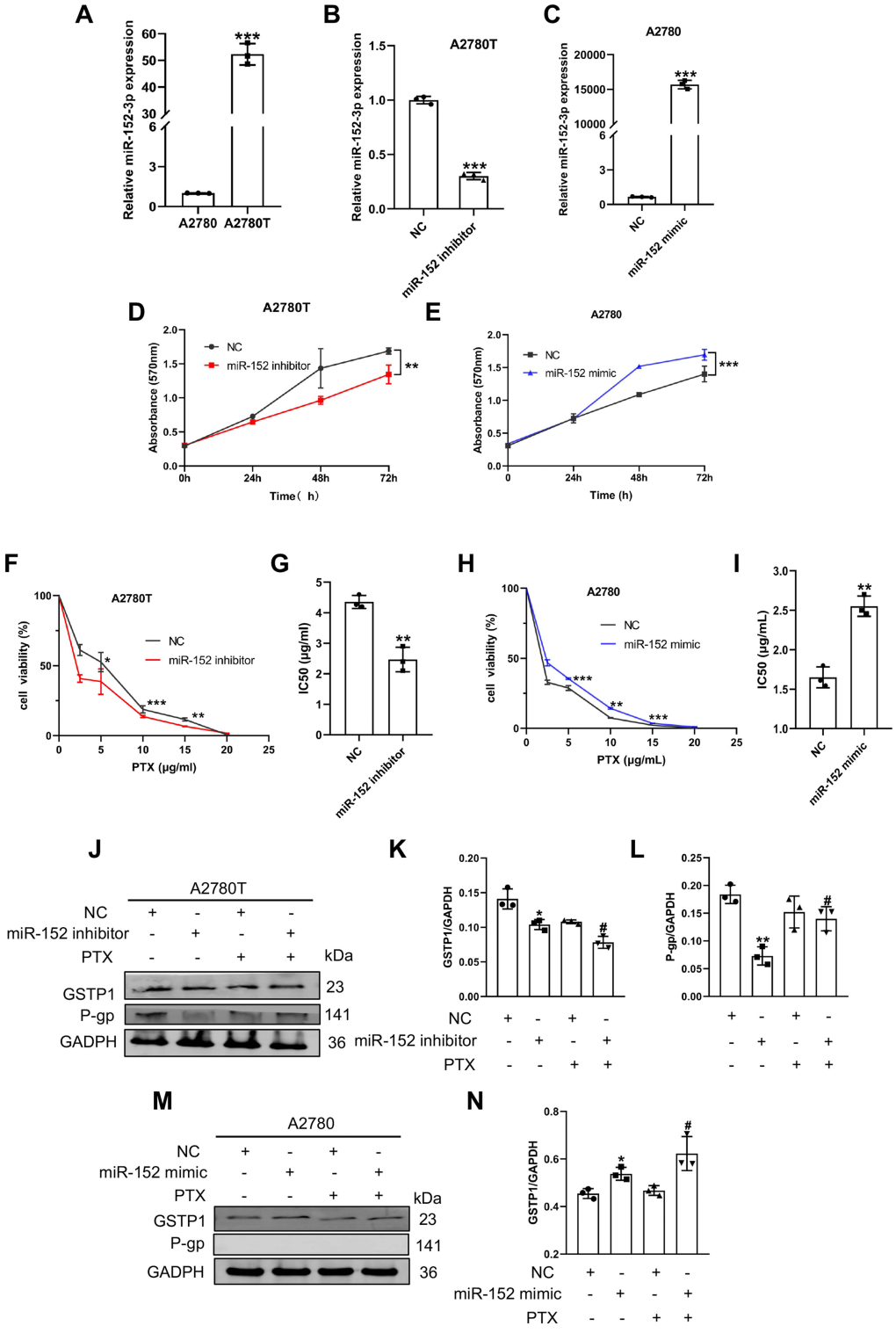

Figure 3 miR-152-3p modulates cell proliferation, apoptosis and paclitaxel sensitivity in ovarian cancer cells. (**A**) Relative miR-152-3p expression in A2780T versus A2780 cells quantified by RT-qPCR. (**B** and **C**) Validation of miR-152-3p inhibition or overexpression 24h post-transfection by RT-qPCR in (**B**) A2780T cells transfected with miR-152-3p inhibitor and (**C**) A2780 cells transfected with miR-152-3p mimic. (**D** and **E**) Cell proliferation assessed in (**D**) A2780T cells transfected with miR-152-3p inhibitor and (**E**) A2780 cells transfected with miR-152-3p mimic. (**F–I**) Cell viability measured by MTT assay after paclitaxel treatment at various concentrations for 48h in transfected cells. (**J–L**) Expression of drug resistance-related proteins P-gp and GSTP1 analyzed by Western blot after miR-152-3p inhibition. (**M** and **N**) P-gp and GSTP1 protein levels after miR-152-3p overexpression. Data are presented as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs NC; $^{\#}P < 0.05$ vs NC+PTX.

Abbreviations: NC, negative control; PTX, paclitaxel; miR, microRNA; SD, standard deviation.

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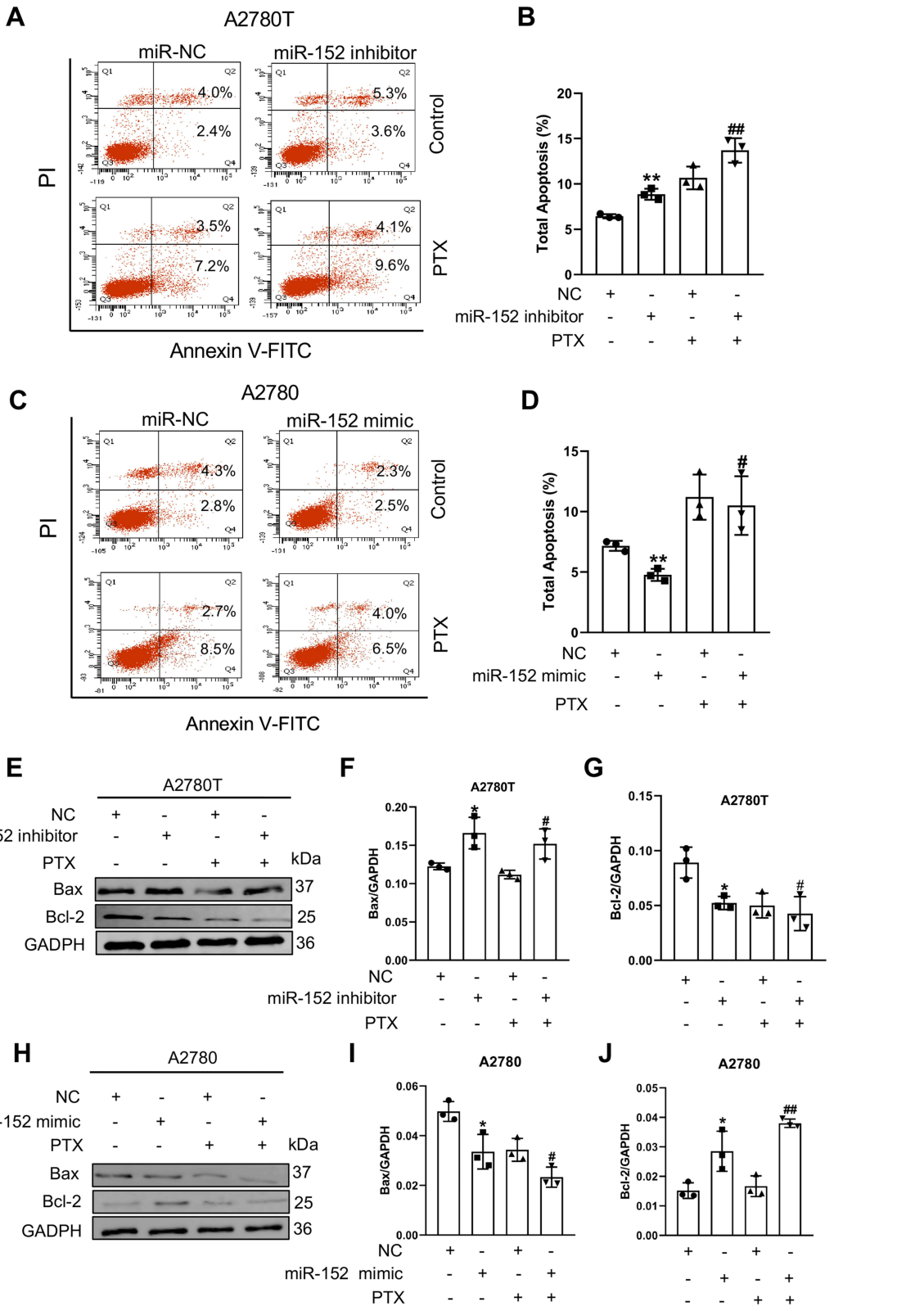

Figure 4 Impact of miR-152-3p on cellular apoptosis. (A and **B**) Flow cytometric analysis of apoptosis in A2780T cells transfected with miR-152-3p inhibitor and treated with paclitaxel (400 ng/mL, 24h). (**C** and **D**) Apoptosis analysis in A2780 cells transfected with miR-152-3p mimic followed by paclitaxel treatment. Apoptotic cells were quantified as the sum of Annexin V-positive and Annexin V/PI double-positive cells. (**E**–**G**) Western blot analysis of apoptosis-related proteins Bax and Bcl-2 in A2780T cells after miR-152-3p inhibition. (**H**–**J**) Bax and Bcl-2 protein levels in A2780 cells following miR-152-3p overexpression. Data represent mean ± SD from three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ vs NC; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs NC+PTX.

Abbreviations: NC, negative control; PTX, paclitaxel; miR, microRNA; ANXA5, annexin V; PI, propidium iodide; SD, standard deviation.

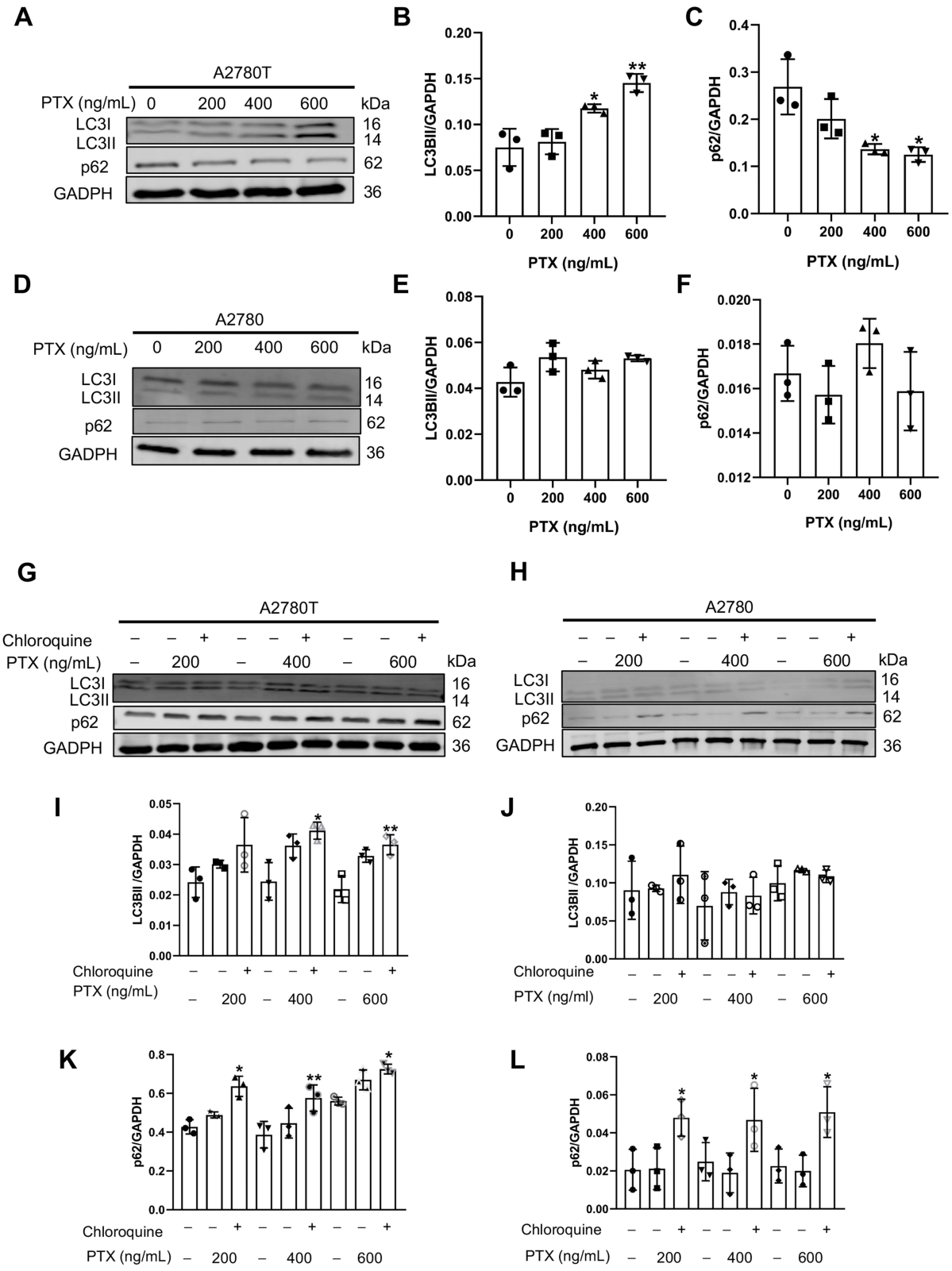

Figure 5 Paclitaxel induces autophagic flux in PTX-resistant cells. (**A**–**C**) Western blot analysis of LC3-II and p62 expression in A2780T cells treated with increasing concentrations of paclitaxel (0–600 ng/mL, 24h). (**D**–**F**) LC3-II and p62 protein levels in A2780 cells under identical treatment conditions. (**G**–**J**) Analysis of autophagic flux in A2780T cells treated with paclitaxel in the presence of chloroquine (20 nM). (**K**–**L**) Corresponding analysis in A2780 cells. Data are presented as mean ± SD. **P* < 0.05, ***P* < 0.01 vs negative control. **Abbreviations**: PTX, paclitaxel; LC3, light chain 3; CQ, chloroquine.

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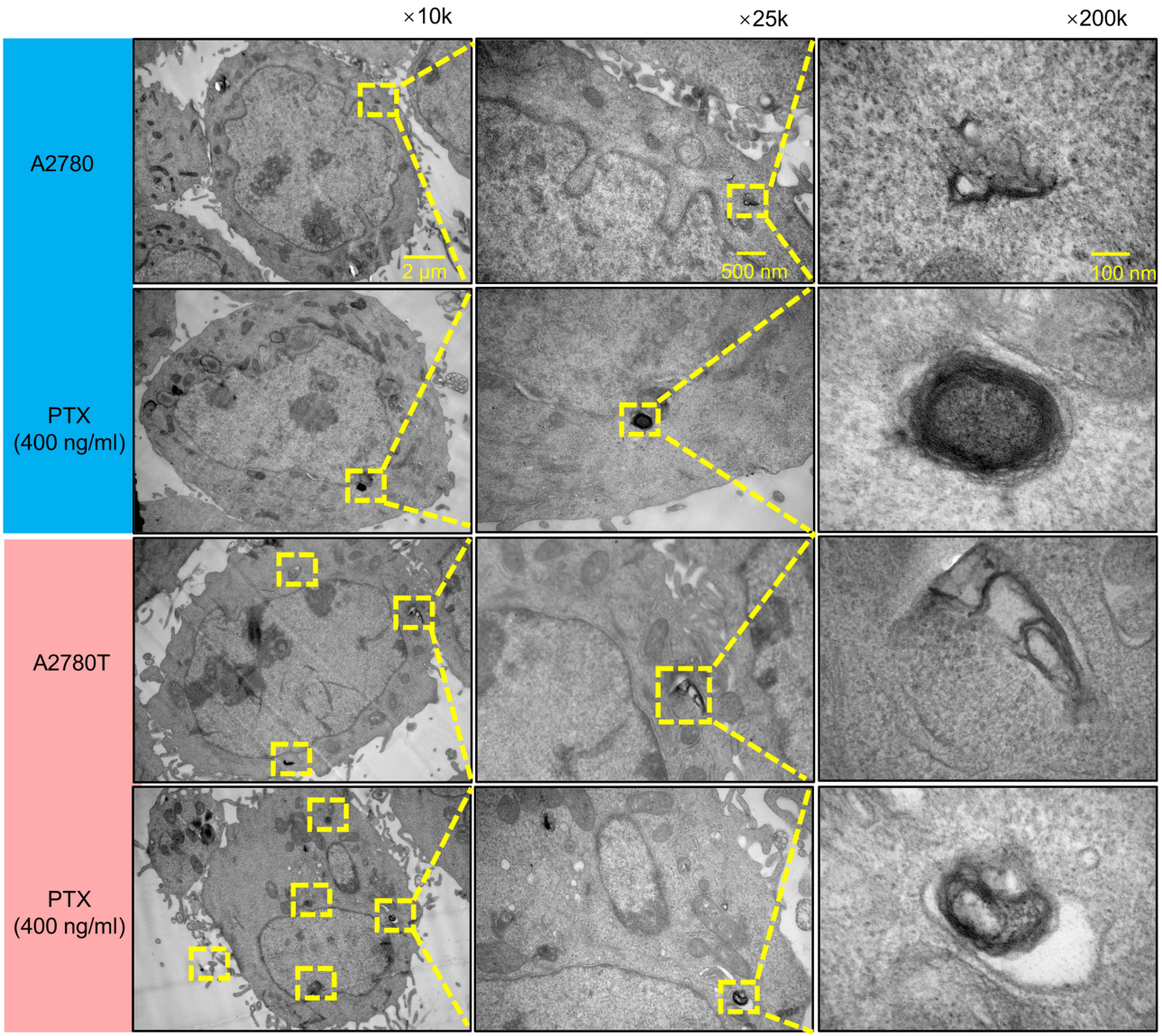

Figure 6 Observation of autophagosomes in A2780 and A2780T cells under transmission electron microscopy (TEM). Yellow boxes indicate autophagosomes, identified by their characteristic double-membrane structures and the presence of engulfed cellular components. The blue area represents A2780 cells treated with 400 ng/mL paclitaxel (PTX) for 24 h, while the pink area represents A2780T cells treated with 400 ng/mL PTX for 24 hours. Images are presented at different magnifications: ×10,000 in the left panel, ×25,000 in the middle panel, and ×200,000 in the right panel. Scale bars: 2 μm in the left panel, 500 nm in the middle panel, and 100 nm in the right panel.
Abbreviation: PTX, paclitaxel.

To elucidate the mechanism by which miR-152-3p regulates autophagy, we conducted comprehensive bioinformatic analyses using multiple prediction tools (TargetScan, miRDB, and miRWalk) integrated with the Human Autophagy Database (HADb). This analysis identified four autophagy-related genes as potential targets: ATG4D, PTEN, CANX, and GRID2 [Figure 7D]. Correlation analysis using StarBase v3.0 platform and filtering for $|r| > 0.1$ further narrowed our focus to ATG4D and PTEN [Figure 7E].

The ATG4 family plays a role in autophagosome biogenesis, transporting contents to lysosomes,[20] and PTEN, a known autophagy regulator, emerged as promising candidates.[31] While PTEN is traditionally considered a tumor suppressor, recent evidence suggests context-dependent oncogenic functions, particularly when modified by Nedd8 and localized to the nucleus.[32] Experimental validation revealed significantly higher expression of both ATG4D and PTEN in A2780T cells compared to A2780 cells [Figure 7F and G]. Notably, miR-152-3p knockdown in A2780T cells led to

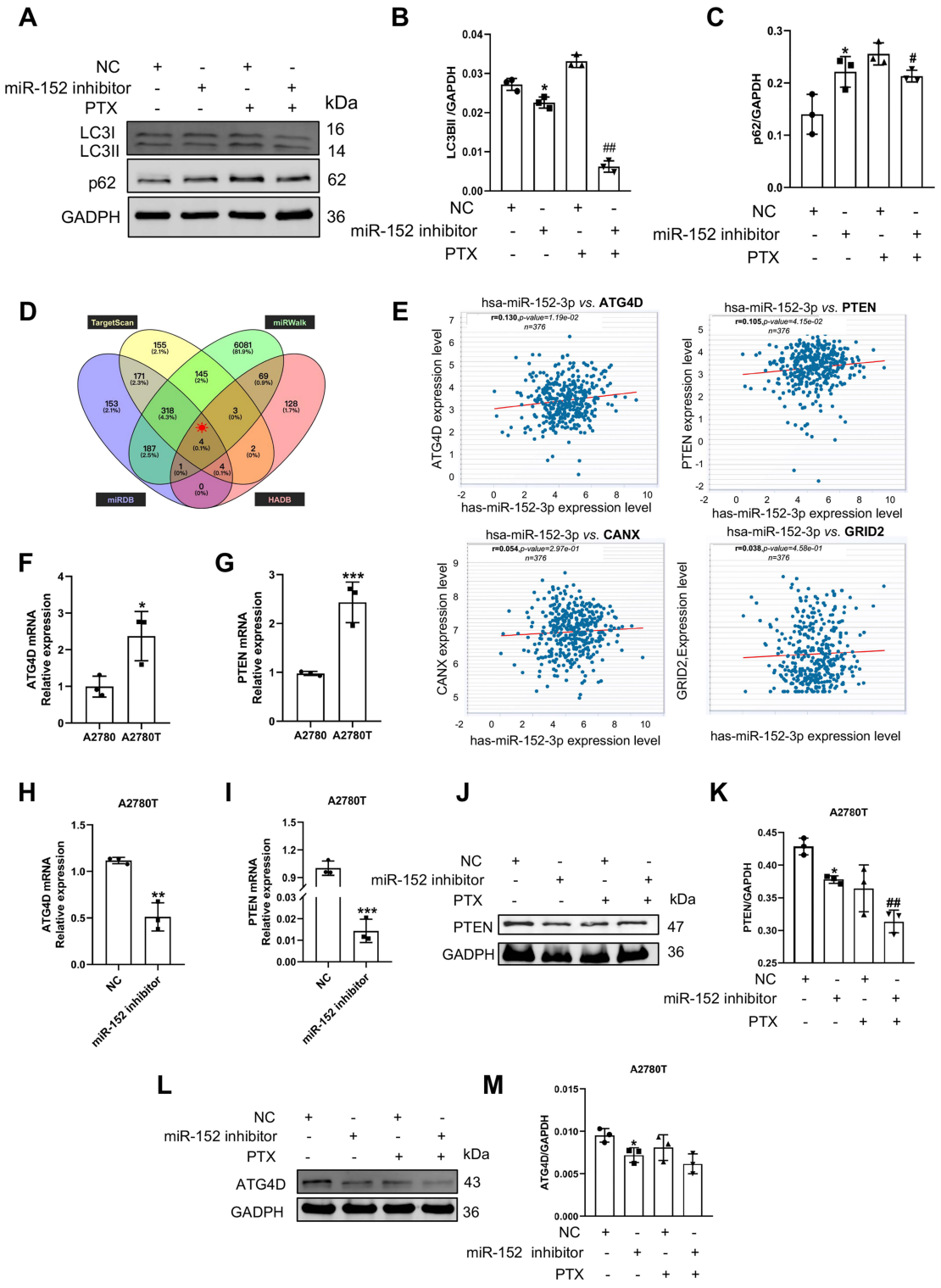

Figure 7 miR-152-3p knockdown suppresses paclitaxel-induced autophagy in A2780T cells, with PTEN and ATG4D identified as miR-152-3p target genes. (**A**–**C**) After silencing of miR-152-3p, the changes in expression of the autophagy-related proteins LC3II and p62 were detected by Western blot. Data shown in (**B**) and (**C**) represent quantification from three independent biological replicates (n=3). The data are presented as the mean ± standard deviation. (**D**) Venn diagram showing the intersection of predicted miR-152-3p target genes across four databases (Human Autophagy Database, TargetScan, miRDB, and miRWalk), identifying ATG4D, PTEN, CANX, and GRID2 as common targets. (**E**) Correlation analysis between miR-152-3p expression and ATG4D, PTEN, CANX, and GRID2 expression levels using the starBase platform. (**F** and **G**) RT-qPCR analysis comparing ATG4D and PTEN expression levels between A2780T and A2780 cells. (**H** and **I**) RT-qPCR validation of the regulatory relationship between miR-152-3p and its targets ATG4D and PTEN. (**J**–**M**) Western blot analysis showing PTEN and ATG4D protein levels following indicated treatments. Data are presented as mean ± standard deviation. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs NC; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs NC + PTX.

Abbreviations: NC, negative control; miR, microRNA; PTX, paclitaxel; r, correlation coefficient; ATG4D, autophagy related 4D cysteine peptidase; PTEN, phosphatase and tensin homolog; CANX, calnexin; GIRD2, glutamate ionotropic receptor δ type subunit 2.

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decreased expression of both ATG4D and PTEN at both mRNA and protein levels [Figure 7H–M], suggesting an indirect positive regulatory relationship between miR-152-3p and these autophagy-related genes.

PTEN Knockdown Enhances PTX Sensitivity in A2780T Cells

High expression of PTEN in A2780T cells was verified in [Figure 7], and changes in IC_{50} and drug resistance proteins were evaluated by silencing the expression of miR-152-3p in A2780T cells. Transfection of PTEN-specific siRNA achieved approximately 60% reduction in PTEN expression compared to the negative control, as verified by RT-qPCR [Figure 8A]. MTT assays demonstrated that PTEN silencing significantly decreased the IC50 value of PTX in A2780T cells [Figure 8B and C]. Western blot analysis revealed concurrent downregulation of multiple drug resistance markers, including P-glycoprotein (P-gp), multidrug resistance protein 1 (MDR1), and ATP-binding cassette subfamily G member 2 (ABCG2) [Figure 8D–F]. These findings indicate that PTEN depletion resensitizes resistant ovarian cancer cells to PTX treatment.

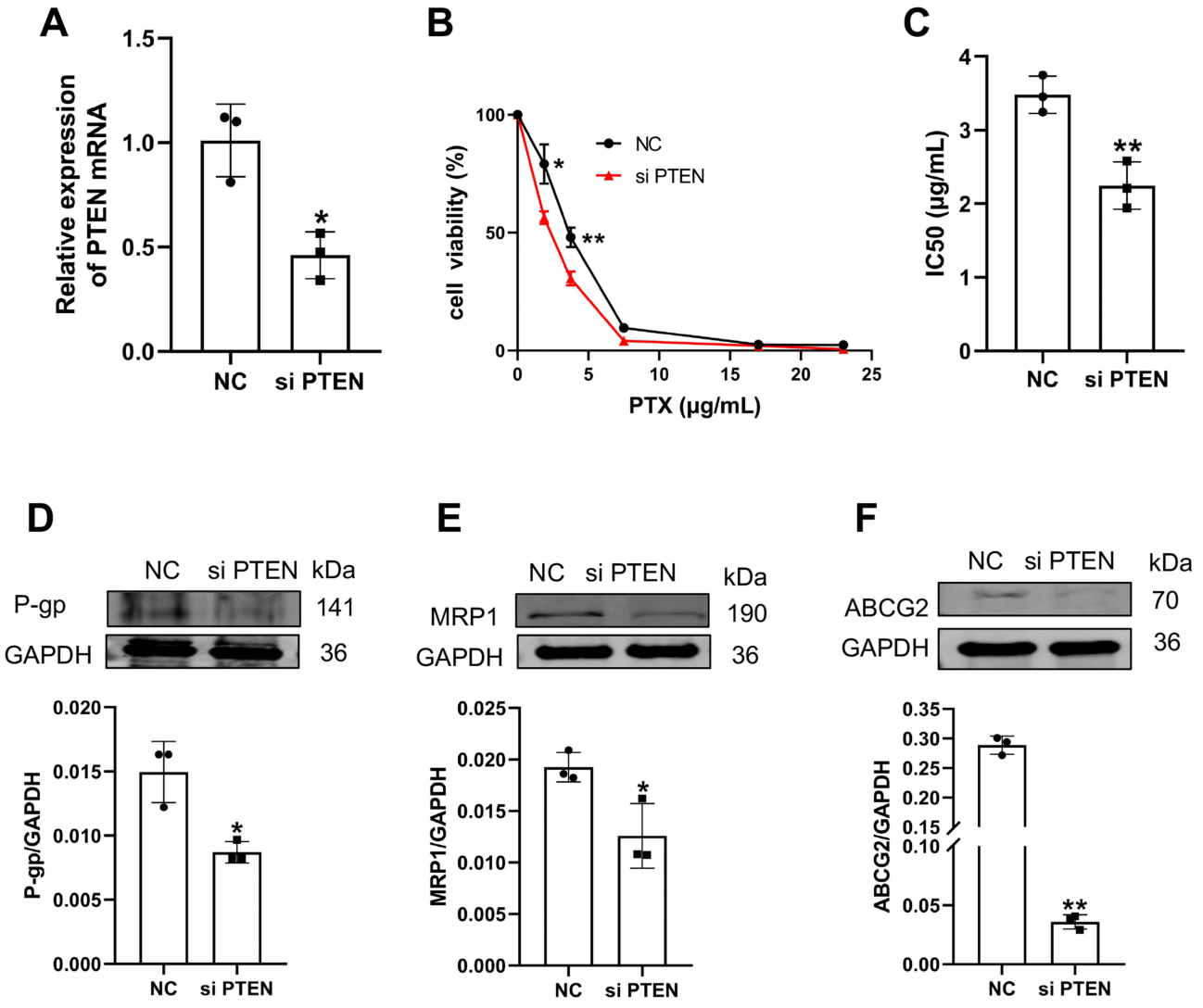

Figure 8 PTEN knockdown enhances PTX sensitivity in A2780T cells. (**A**) PTEN knockdown efficiency validated by RT-qPCR. (**B** and **C**) Cell viability and IC50 values assessed by MTT assay following PTEN silencing. (**D**–**F**) Expression analysis of drug resistance-associated proteins by Western blot after PTEN knockdown. Data are presented as mean ± SD. **P* < 0.05, ***P* < 0.01 vs NC.

Abbreviations: PTEN, phosphatase and tensin homolog; NC, negative control.

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Discussion

The miR-148/152 family, including miR-148a, miR-148b and miR-152, are involved in tumorigenesis and tumor progression,[33] while the role and drug resistance mechanism of miR-152-3p have been reported in various cancers such as gastrointestinal cancer, endometrial cancer, liver cancer, breast cancer, prostate cancer and ovarian cancer.[34–37] miR-152-3p was upregulated in ovarian cancer and was correlated with malignant clinicopathological phenotype and overall survival, suggesting that miR-152-3p may become a novel marker for predicting ovarian cancer prognosis.[38] Analysis of TCGA, GEO online database found that miR-152-3p was highly expressed in ovarian cancer and PTX-resistant ovarian cancer cells, which was consistent with the results of in vitro experiments. There are limited studies on the mechanism responsible for the participation of miR-152-3p in ovarian cancer's resistance to PTX. Cisplatin and paclitaxel are the first-line chemotherapeutic drugs for ovarian cancer treatment,[39] and previous reports mostly focused on the mechanism of ovarian cancer resistance to cisplatin.[40–43] For example, overexpression of miR-29c-3p partially inhibited autophagy and ovarian cancer resistance to cisplatin by downregulating the forkhead box protein P1/ATG14 pathway, while miR-142-5p inhibited multiple anti-apoptotic genes such as X-linked inhibitor of apoptosis protein (XIAP) by targeting them. While it has been reported that miR-152-3p promotes the apoptosis of ovarian cancer cells,[44] but there is no report on the involvement of miR-152-3p in the resistance mechanism of ovarian cancer to PTX.

In the present study study, online gene databases (miRDB, miRwalk, TargetScan and autophagy-related database HADB) were used to collect miR-152-3p data and predict its autophagy-related target genes. The targeting association of ATG4D, PTEN, CANX, GRID2 and miR-152-3p was predicted through the starBase v3.0 platform, and through the correlation coefficient comparison, genes with a correlation coefficient $|r|$ value >0.1 were selected. Thus, ATG4D and PTEN were identified as the predicted target genes of miR-152-3p, and subsequent biological experiments were conducted according to this theoretical foundation. It was found that, when the expression of miR-152-3p in A2780T cells was inhibited, the expression of ATG4D and PTEN was decreased. Although miRNAs are widely considered to negatively regulate downstream target genes, in vitro experiments have shown that miRNAs can also activate gene expression.[45] G-rich RNA sequence binding factor 1-mediated miR-G-10 positively regulates the level of PIK3R3 and activates the AKT/NF-κB signaling pathway to inhibit the migration and invasion of cervical cancer.[46] miR-G-1 positively regulates the levels of transmembrane P24 trafficking protein 5 and lamin B1-mediated activation of autophagy, which promotes the sensitivity of cervical cancer to PTX.[47] Previous studies have found that PTEN is negatively regulated by miR-152-3p in heart disease, diabetes, non-small cell lung cancer and other diseases.[48–53] However, the targeting relationship of miR-152-3p with PTEN and ATG4D in PTX-resistant ovarian cancer cells has not been reported to date. Previous studies have proposed a potential mechanism for miRNA to positively regulate genes: intragenic miRNA-host gene co-expression; miRNA targets and represses an intermediate gene, which in turn acts as a repressor of another gene related to the miRNA; a gene can act as a co-activator of transcription of miRNAs and target genes; super-enhancer-mediated co-expression of miRNA genes, and direct binding of miRNAs to regulatory regions of partner genes.[54] The potential mechanism of miRNA's positive regulation of genes provides a foundation for our future studies. The present findings indirectly indicate that the expression of PTEN and ATG4D may be positively regulated by miR-152-3p, although the specific regulatory association and potential mechanism need to be further verified by dual-luciferase reporter assay in future studies.

Autophagy is a survival adaptation mechanism that degrades excess or damaged organelles, large protein aggregates, and invading pathogens through the lysosomal system (vacuoles in plants and yeast), and is generally induced by stress stimuli such as hypoxia, energy or amino acid deprivation, radiation, drugs, etc. Autophagy plays an important role in the development of drug resistance in ovarian cancer therapy, and inhibition of autophagy enhances the antitumor effect of anticancer drugs on ovarian cancer.[55,56] Therefore, an ideal anti-ovarian cancer drug should inhibit both cell proliferation and autophagy to reduce the resistance of ovarian cancer to chemotherapeutic drugs.[57–60] ATG4D is a core protein of autophagy, and has been reported to play a role between autophagy and apoptosis.[61] However, the specific effects of ATG4D on malignancies, including ovarian cancer, remain unclear. A previous study has reported that silencing ATG4D can significantly inhibit cell proliferation, promote cell apoptosis, and enhance the sensitivity of hepatocellular carcinoma to cisplatin, indicating that ATG4D can maintain the survival of cancer cells.[62] PTEN is a tumor suppressor that is

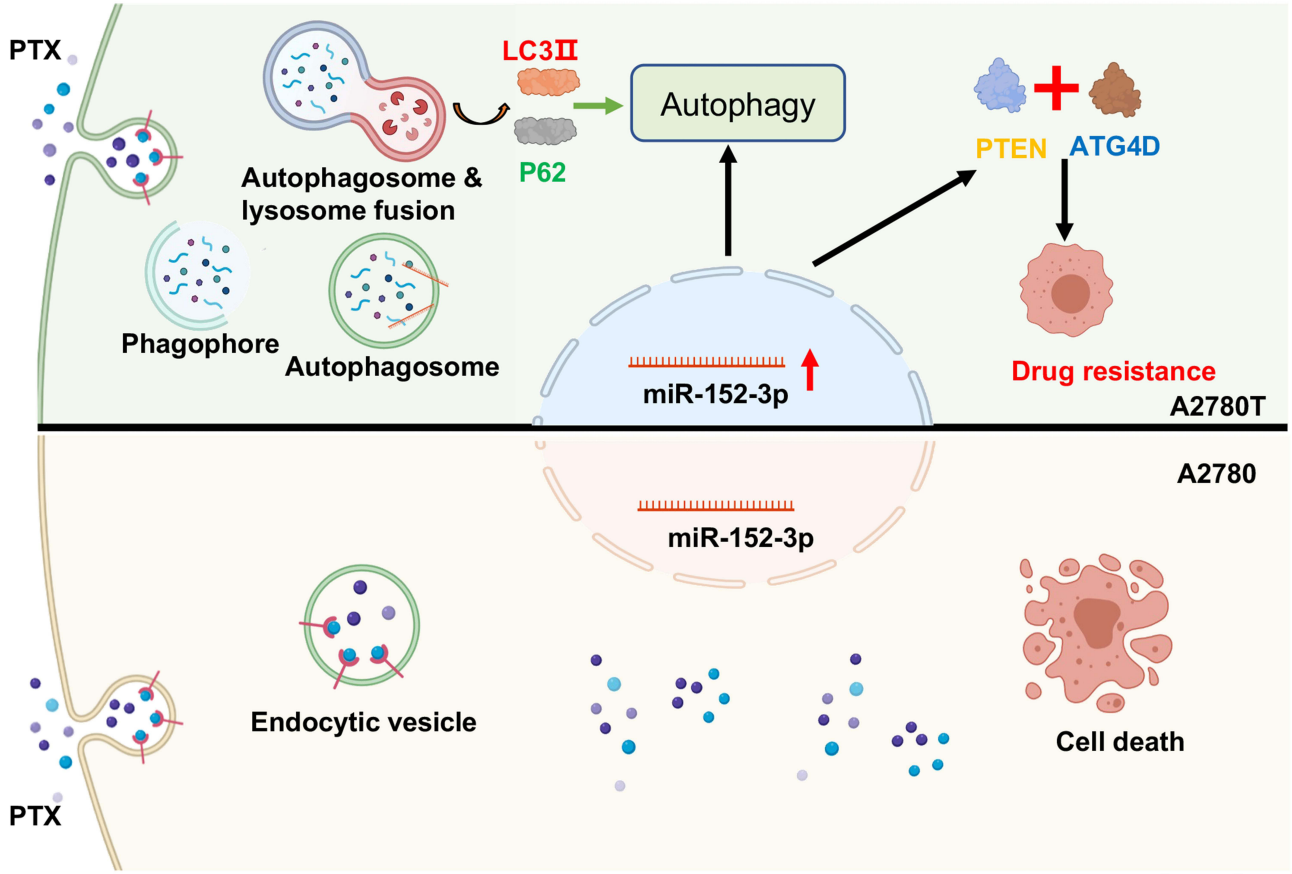

Figure 9 Proposed mechanism of miR-152-3p-mediated paclitaxel resistance through PTEN-dependent autophagy regulation in ovarian cancer cells. In paclitaxel-resistant ovarian cancer cells (A2780T), elevated miR-152-3p expression and paclitaxel-induced autophagy are observed compared to sensitive cells (A2780). Bioinformatic analysis identified PTEN and ATG4D (autophagy related 4D cysteine peptidase) as autophagy-related target genes of miR-152-3p. miR-152-3p inhibition in A2780T cells led to increased PTEN expression, while PTEN silencing reduced both drug resistance-related protein expression and IC50 values. Furthermore, miR-152-3p downregulation suppressed cell proliferation, enhanced apoptosis, and decreased IC50 values. These findings demonstrate that miR-152-3p inhibition reverses paclitaxel resistance by targeting PTEN to suppress autophagy in paclitaxel-resistant ovarian cancer cells.
Abbreviations: PTX, paclitaxel; miR, microRNA; PTEN, phosphatase and tensin homolog; IC50, half-maximal inhibitory concentration.

frequently mutated in human cancers, and it has been reported that PTEN can promote cancer in the nucleus, and has multiple cytoplasmic and nuclear functions.[63] PTEN induces autophagy and is associated with activation of the p-JUN-SESN2/AMPK pathway.[64] A previous study found that the autophagy protein Beclin-1 may interact with PTEN to participate in drug resistance mechanisms and changes in macrophage activity observed in drug-resistant ovarian cancer.[65] This study identifies miR-152-3p and PTEN as promising therapeutic targets.[66] However, the roles and molecular mechanisms of ATG4D and PTEN in PTX-sensitive and drug-resistant ovarian cancer cells have not been reported to date. To further explore whether miR-152-3p can regulate autophagy by targeting PTEN to reverse the resistance of PTX-resistant cells to this drug, it is necessary to co-transfect miR-152-3p and PTEN in A2780T cells. After knockdown of PTEN in PTX-resistant ovarian cancer cells, both the resistance protein and IC_{50} were decreased, suggesting that PTEN may be a potential target for clinical treatment of ovarian cancer. While P-glycoprotein (P-gp) is well-established as the primary efflux transporter involved in paclitaxel resistance,[67] we observed changes in MRP1 and ABCG2 expression following PTEN silencing. Although paclitaxel may not be a direct substrate for MRP1 and ABCG2, these transporters have been implicated in multidrug resistance mechanisms through indirect pathways.[24,68] The decreased expression of these transporters following PTEN silencing may reflect broader changes in cellular drug resistance mechanisms rather than direct paclitaxel transport, as PTEN has been shown to regulate multiple drug resistance pathways through PI3K/AKT signaling.[69,70] Previous studies have demonstrated that PTEN can modulate ABC transporter expression and activity through the PI3K/AKT pathway,[71] suggesting that PTEN's role in drug

resistance involves complex regulatory networks beyond direct regulation of specific drug transporters. Future studies are needed to elucidate the precise relationship between PTEN regulation and the expression of these drug resistance-associated proteins in ovarian cancer chemoresistance.

Conclusion

The present study highlighted the biological significance of miR-152-3p in ovarian cancer, and elucidates the molecular mechanism of PTX-resistant cells. Low levels of miR-152-3p reversed PTX resistance in PTX-resistant cells by targeting PTEN to inhibit autophagy [Figure 9]. The validation of these findings and their translational relevance in overcoming chemotherapy resistance would be achieved through the exploration of in vivo models and clinical samples. These findings provide new therapeutic targets and prognostic markers for individualized treatment of ovarian cancer. Future translation studies based on miR-152-3p postoperative trials and therapeutic interventions will benefit patients with ovarian cancer.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

Authors state no conflict of interest.

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References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin*. 2022;72(1):7–33. doi:10.3322/caac.21708
2. Berek JS, Bertelsen K, du Bois A, et al. Advanced epithelial ovarian cancer: 1998 consensus statements. *Ann Oncol*. 1999;10 Suppl 1:87–92. doi:10.1023/a:1008323922057
3. Kuroki L, Guntupalli SR. Treatment of epithelial ovarian cancer. *BMJ*. 2020;371:m3773. doi:10.1136/bmj.m3773
4. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. *Cell*. 2008;132(1):27–42. doi:10.1016/j.cell.2007.12.018
5. Mathew R, Karantza-Wadsworth V, White E. Role of autophagy in cancer. *Nat Rev Cancer*. 2007;7(12):961–967. doi:10.1038/nrc2254
6. Perrotta C, Cattaneo MG, Molteni R, De Palma C. Autophagy in the Regulation of Tissue Differentiation and Homeostasis. *Front Cell Dev Biol*. 2020;8(602901). doi:10.3389/fcell.2020.602901
7. Li X, Liang X, Yin J, Lin W. Organic fluorescent probes for monitoring autophagy in living cells. *Chem Soc Rev*. 2021;50(1):102–119. doi:10.1039/d0cs00896f
8. Du H, Yang W, Chen L, et al. Role of autophagy in resistance to oxaliplatin in hepatocellular carcinoma cells. *Oncol Rep*. 2012;27(1):143–150. doi:10.3892/or.2011.1464
9. Verma AK, Bharti PS, Rafat S, et al. Autophagy Paradox of Cancer: role, Regulation, and Duality. *Oxid Med Cell Longev*. 2021;2021(8832541). doi:10.1155/2021/8832541
10. SF Zhang, XY Wang, ZQ Fu, et al. TXNDC17 promotes paclitaxel resistance via inducing autophagy in ovarian cancer. *Autophagy*. 2015;11(2):225–238. doi:10.1080/15548627.2014.998931
11. Worby CA, Dixon JE. Reversible phosphorylation: a birthday tribute to Herb Tabor. *J Biol Chem*. 2019;294(5):1638–1642. doi:10.1074/jbc.TME118.004161
12. Wu H, Wang K, Liu W, Hao Q. PTEN overexpression improves cisplatin-resistance of human ovarian cancer cells through upregulating KRT10 expression. *Biochem Biophys Res Commun*. 2014;444(2):141–146. doi:10.1016/j.bbrc.2014.01.014
13. YING H, QU D, LIU C. Chemoresistance is associated with Beclin-1 and PTEN expression in epithelial ovarian cancers. *Oncol Lett*. 2015;9(4):1759–1763. doi:10.3892/ol.2015.2950
14. Wu Y, Xu W, Yang Y, Zhang Z. miRNA-93-5p Promotes Gemcitabine Resistance in Pancreatic Cancer Cells by Targeting the PTEN-Mediated PI3K/Akt Signaling Pathway. *Ann Clin Lab Sci*. 2021;51(3):310–320.
15. Xu WP, Liu JP, Feng JF, et al. miR-541 potentiates the response of human hepatocellular carcinoma to sorafenib treatment by inhibiting autophagy. *Gut*. 2020;69(7):1309–1321. doi:10.1136/gutjnl-2019-318830

16. Huang T, Wan X, Alvarez AA. MIR93 (microRNA -93) regulates tumorigenicity and therapy response of glioblastoma by targeting autophagy. *Autophagy*. 2019;15(6):1100–1111. doi:10.1080/15548627.2019.1569947
17. Kang -Y-Y, Liu Y, Wang M-L. Construction and analyses of the microRNA-target gene differential regulatory network in thyroid carcinoma. *PLoS One*. 2017;12(6):e0178331. doi:10.1371/journal.pone.0178331
18. Loo WT, Fong JH, Cheung MN, Chow LW. The efficacy of Paclitaxel on solid tumour analysed by ATP bioluminescence assay and VEGF expression: a translational research study. *Biomed Pharmacother*. 2005;59(2):S337–9. doi:10.1016/s0753-3322(05)80069-1
19. de Mello GH, D'ávila da Silva CM, Viana AR, Krause LMF, Cadoná FC. Cocoa presents cytotoxicity against melanoma cancer cell lines (A-375 e B16-F10) and improves chemotherapy activity by increasing oxidative stress. *J Food Biochem*. 2022;46(12):e14512. doi:10.1111/jfbc.14512
20. Tamargo-Gómez I, Martínez-García GG, Suárez MF. ATG4D is the main ATG8 delipidating enzyme in mammalian cells and protects against cerebellar neurodegeneration. *Cell Death Differ*. 2021;28(9):2651–2672. doi:10.1038/s41418-021-00776-1
21. Lanci A, Ingrà L, Dondi F. Morphological study of equine amniotic compartment. *Theriogenology*. 2022;177:165–171. doi:10.1016/j.theriogenology.2021.10.019
22. Zhao C, Qiu L, Wu D, et al. Targeted reversal of multidrug resistance in ovarian cancer cells using exosome-encapsulated tetramethylpyrazine. *Mol Med Rep*. 2024;29(2):25. doi:10.3892/mmr.2023.13148
23. Li Y, Zhou D, Ren Y. Mir223 restrains autophagy and promotes CNS inflammation by targeting ATG16L1. *Autophagy*. 2019;15(3):478–492. doi:10.1080/15548627.2018.1522467
24. Robey RW, Pluchino KM, Hall MD, Fojo AT, Bates SE, Gottesman MM. Revisiting the role of ABC transporters in multidrug-resistant cancer. *Nat Rev Cancer*. 2018;18(7):452–464. doi:10.1038/s41568-018-0005-8
25. Singh SK, Bahal R, Rasmussen TP. Evidence that miR-152-3p is a positive regulator of SETDB1-mediated H3K9 histone methylation and serves as a toggle between histone and DNA methylation. *Exp Cell Res*. 2020;395(2):112216. doi:10.1016/j.yexcr.2020.112216
26. Norouzi-Barough L, Sarookhani MR, Sharifi M, Moghbelinejad S, Jangjoo S, Salehi R. Molecular mechanisms of drug resistance in ovarian cancer. *J Cell Physiol*. 2018;233(6):4546–4562. doi:10.1002/jcp.26289
27. Zhao H, Li R, Wang X. Retraction Note: the role of apatinib combined with paclitaxel (aluminum binding type) in platinum-resistant ovarian cancer. *J Ovarian Res*. 2021;14(1):37. doi:10.1186/s13048-021-00785-1
28. Yu F, Dong B, Dong P, He Y, Zheng J, Xu P. Hypoxia induces the activation of hepatic stellate cells through the PVT1-miR-152-ATG14 signaling pathway. *mol Cell Biochem*. 2020;465(1–2):115–123. doi:10.1007/s11010-019-03672-y
29. Kirisako T, Ichimura Y, Okada H. The reversible modification regulates the membrane-binding state of Apg8/Aut7 essential for autophagy and the cytoplasm to vacuole targeting pathway. *J Cell Biol*. 2000;151(2):263–276. doi:10.1083/jcb.151.2.263
30. Bjørkøy G, Lamark T, Brech A, et al. p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. *J Cell Biol*. 2005;171(4):603–614. doi:10.1083/jcb.200507002
31. Álvarez-Garcia V, Tawil Y, Wise HM, Leslie NR. Mechanisms of PTEN loss in cancer: it's all about diversity. *Semin Cancer Biol*. 2019;59:66–79. doi:10.1016/j.semcancer.2019.02.001
32. Xie P, Peng Z, Chen Y. Neddylation of PTEN regulates its nuclear import and promotes tumor development. *Cell Res*. 2021;31(3):291–311. doi:10.1038/s41422-020-00443-z
33. Friedrich M, Pracht K, Mashreghi M-F, Jäck H-M, Radbruch A, Seliger B. The role of the miR-148/-152 family in physiology and disease. *Eur J Immunol*. 2017;47(12):2026–2038. doi:10.1002/eji.201747132
34. Xu -S-S, Ding J-F, Shi P, Shi K-H, Tao H. DNMT1-Induced miR-152-3p Suppression Facilitates Cardiac Fibroblast Activation in Cardiac Fibrosis. *Cardiovasc Toxicol*. 2021;21(12):984–999. doi:10.1007/s12012-021-09690-x
35. Li H, Liu D, Liu L, Huang S, Ma A, Zhang X. The role of HOTAIR/miR-152-3p/LIN28B in regulating the progression of endometrial squamous carcinoma. *Arch Med Sci*. 2021;17(2):434–448. doi:10.5114/aoms.2019.89632
36. Zhao Z, Wu C, He X, et al. miR-152-3p aggravates vascular endothelial cell dysfunction by targeting DEAD-box helicase 6 (DDX6) under hypoxia. *Bioengineered*. 2021;12(1):4899–4910. doi:10.1080/21655979.2021.1959864
37. Fan Y, Gan M, Tan Y, et al. Mir-152 Regulates 3T3-L1 Preadipocyte Proliferation and Differentiation. *Molecules*. 2019;24(18):3379. doi:10.3390/molecules24183379
38. Khajehnoori S, Zarei F, Mazaheri M, Dehghani-Firoozabadi A. Epidrug Modulated Expression of MiR--152 and MiR-148a Reverse Cisplatin Resistance in Ovarian Cancer Cells: an Experimental In-vitro Study. *Iran J Pharm Res*. 2020;19(3):509–519. doi:10.22037/ijpr.2020.15450.13217
39. Falcetta F, Bizzaro F, D'Agostini E, Bani MR, Giavazzi R, Ubezio P. Modeling Cytostatic and Cytotoxic Responses to New Treatment Regimens for Ovarian Cancer. *Cancer Res*. 2017;77(23):6759–6769. doi:10.1158/0008-5472.CAN-17-1099
40. Liu R, Zhang Y, Sun P, Wang C. Retraction to 'DDP-resistant ovarian cancer cells-derived exosomal microRNA-30a-5p reduces the resistance of ovarian cancer cells to DDP'. *Open Biol*. 2021;11(11):210329. doi:10.1098/rsob.210329
41. Liu R, Zhang Y, Sun P, Wang C. Expression of concern: DDP-resistant ovarian cancer cells-derived exosomal microRNA-30a-5p reduces the resistance of ovarian cancer cells to DDP. *Open Biol*. 2021;11(10):210301. doi:10.1098/rsob.210301
42. Cao Y, Xie X, Li M, Gao Y. CircHIPK2 Contributes to DDP Resistance and Malignant Behaviors of DDP-Resistant Ovarian Cancer Cells Both in vitro and in vivo Through circHIPK2/miR-338-3p/CHTOP ceRNA Pathway. *Onco Targets Ther*. 2021;14:3151–3165. doi:10.2147/OTT.S291823
43. Zhong Y, Le F, Cheng J. Triptolide inhibits JAK2/STAT3 signaling and induces lethal autophagy through ROS generation in cisplatin-resistant SKOV3/DDP ovarian cancer cells. *Oncol Rep*. 2021;45(5):1. doi:10.3892/or.2021.8020
44. Li X, Chen W, Jin Y, et al. miR-142-5p enhances cisplatin-induced apoptosis in ovarian cancer cells by targeting multiple anti-apoptotic genes. *Biochem Pharmacol*. 2019;161:98–112. doi:10.1016/j.bcp.2019.01.009
45. Shi X, Liu -T-T, Yu X-N, et al. microRNA-93-5p promotes hepatocellular carcinoma progression via a microRNA-93-5p/MAP3K2/c-Jun positive feedback circuit. *Oncogene*. 2020;39(35):5768–5781. doi:10.1038/s41388-020-01401-0
46. Sun Q, Yang Z, Li P. A novel miRNA identified in GRSF1 complex drives the metastasis via the PIK3R3/AKT/NF-κB and TIMP3/MMP9 pathways in cervical cancer cells. *Cell Death Dis*. 2019;10(9):636. doi:10.1038/s41419-019-1841-5
47. Yang Z, Sun Q, Guo J. GRSF1-mediated MIR-G-1 promotes malignant behavior and nuclear autophagy by directly upregulating TMED5 and LMNB1 in cervical cancer cells. *Autophagy*. 2019;15(4):668–685. doi:10.1080/15548627.2018.1539590
48. Li B, Luan S, Chen J. The MSC-Derived Exosomal lncRNA H19 Promotes Wound Healing in Diabetic Foot Ulcers by Upregulating PTEN via MicroRNA-152-3p. *mol Ther Nucleic Acids*. 2020;19:814–826. doi:10.1016/j.omtn.2019.11.034

49. Xu Y, Yu T, He L. Inhibition of miRNA-152-3p enhances diabetic wound repair via upregulation of PTEN. *Aging*. 2020;12(14):14978–14989. doi:10.18632/aging.103557
50. Zhai K, Liu B, Gao L. Promotes Parkinson's Disease via Modulating Pathway. *Hum Gene Ther*. 2020;31(23–24):1274–1287. doi:10.1089/hum.2020.106
51. Zhang H, Zhang H, Li X, Huang S, Guo Q, Geng D. LINC01089 functions as a ceRNA for miR-152-3p to inhibit non-small lung cancer progression through regulating PTEN. *Cancer Cell Int*. 2021;21(1):143. doi:10.1186/s12935-021-01846-7
52. Świderska E, Strycharz J, Wróblewski A, et al. Chronic and Intermittent Hyperglycemia Modulates Expression of Key Molecules of PI3K/AKT Pathway in Differentiating Human Visceral Adipocytes. *Int J mol Sci*. 2021;22(14):7712. doi:10.3390/ijms22147712
53. Liu P, Wu L, Chand H, et al. Silencing of miR-152 contributes to DNMT1-mediated CpG methylation of the PTEN promoter in bladder cancer. *Life Sci*. 2020;261:118311. doi:10.1016/j.lfs.2020.118311
54. Tan H, Huang S, Zhang Z, Qian X, Sun P, Zhou X. Pan-cancer analysis on microRNA-associated gene activation. *EBioMedicine*. 2019;43:82–97. doi:10.1016/j.ebiom.2019.03.082
55. Shen -H-H, Zhang T, Yang H-L. Ovarian hormones-autophagy-immunity axis in menstruation and endometriosis. *Theranostics*. 2021;11(7):3512–3526. doi:10.7150/thno.55241
56. Galluzzi L, Green DR. Autophagy-Independent Functions of the Autophagy Machinery. *Cell*. 2019;177(7):1682–1699. doi:10.1016/j.cell.2019.05.026
57. Zhu H, Gan X, Jiang X, Diao S, Wu H, Hu J. ALKBH5 inhibited autophagy of epithelial ovarian cancer through miR-7 and BCL-2. *J Exp Clin Cancer Res*. 2019;38(1):163. doi:10.1186/s13046-019-1159-2
58. Qi G, Ma H, Li Y, Peng J, Chen J, Kong B. TTK inhibition increases cisplatin sensitivity in high-grade serous ovarian carcinoma through the mTOR/autophagy pathway. *Cell Death Dis*. 2021;12(12):1135. doi:10.1038/s41419-021-04429-6
59. Vianello C, Cocetta V, Catanzaro D. Cisplatin resistance can be curtailed by blunting Bnip3-mediated mitochondrial autophagy. *Cell Death Dis*. 2022;13(4):398. doi:10.1038/s41419-022-04741-9
60. Bi J, Zhang Y, Malmrose PK. Blocking autophagy overcomes resistance to dual histone deacetylase and proteasome inhibition in gynecologic cancer. *Cell Death Dis*. 2022;13(1):59. doi:10.1038/s41419-022-04508-2
61. Andaloussi AE, Habib S, Soylemes G, et al. Defective expression of ATG4D abrogates autophagy and promotes growth in human uterine fibroids. *Cell Death Discov*. 2017;3:17041. doi:10.1038/cddiscovery.2017.41
62. Zhao J-Y, Li X-Y, Liu T-D, Liang B, Huang Y, Li W. Silencing of ATG4D suppressed proliferation and enhanced cisplatin-induced apoptosis in hepatocellular carcinoma through Akt/Caspase-3 pathway. *mol Cell Biochem*. 2021;476(11):4153–4159. doi:10.1007/s11010-021-04224-z
63. Kang X, Li C, Xie Y. Hippocampal ornithine decarboxylase/spermidine pathway mediates HS-alleviated cognitive impairment in diabetic rats: involving enhancment of hippocampal autophagic flux. *J Adv Res*. 2021;27:31–40. doi:10.1016/j.jare.2020.06.007
64. Kim SM, Nguyen TT, Ravi A. PTEN Deficiency and AMPK Activation Promote Nutrient Scavenging and Anabolism in Prostate Cancer Cells. *Cancer Discov*. 2018;8(7):866–883. doi:10.1158/2159-8290.CD-17-1215
65. Wani A, Gupta M, Ahmad M. Alborixin clears amyloid-β by inducing autophagy through PTEN-mediated inhibition of the AKT pathway. *Autophagy*. 2019;15(10):1810–1828. doi:10.1080/15548627.2019.1596476
66. Tan H, Hu J, Liu S. Efficacy and safety of nanoparticle albumin-bound paclitaxel in non-small cell lung cancer: a systematic review and meta-analysis. *Artif Cells Nanomed Biotechnol*. 2019;47(1):268–277. doi:10.1080/21691401.2018.1552595
67. Kathawala RJ, Gupta P, Ashby Jr CR, Chen ZS. The modulation of ABC transporter-mediated multidrug resistance in cancer: a review of the past decade. *Drug Resist Updat*. 2015;18:1–17. doi:10.1016/j.drup.2014.11.002
68. Mo W, Zhang JT. Human ABCG2: structure, function, and its role in multidrug resistance. *Int J Biochem mol Biol*. 2012;3(1):1–27.
69. Liu R, Chen Y, Liu G, et al. PI3K/AKT pathway as a key link modulates the multidrug resistance of cancers. *Cell Death Dis*. 2020;11(9):020–02998.
70. Dong C, Wu J, Chen Y, et al. Activation of PI3K/AKT/mTOR Pathway Causes Drug Resistance in Breast Cancer. *Front Pharmacol*. 2021;12:628690.
71. Bleau A-M, Hambardzumyan D, Ozawa T. PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma tumor stem-like cells. *Cell Stem Cell*. 2009;4(3):226–235. doi:10.1016/j.stem.2009.01.007